

We claim:

1. A method of measuring the ability of a test compound to inactivate a biological target in a cell of a subject, comprising the steps of:
  - 5 (a) administering the test compound to a subject, such that any of the biological target in the subject's body which reacts with the test compound is inactivated and any of the biological target which does not react with the test compound is free;
  - (b) removing a biological sample comprising one or more cell types from the subject;
  - (c) determining the amount of free biological target within the biological sample or a 10 fraction thereof; and
  - (d) comparing the amount determined in step (c) with the amount of free biological target in a control sample,  
wherein a decrease in the amount of free biological target determined in step (c) compared to the amount determined in the control sample provides a measure of the 15 amount of inactivated biological target in the biological sample or fraction thereof.
2. The method of Claim 1 wherein the amount of free biological target is determined by measuring the activity of the biomolecule within the biological sample or fraction thereof.  
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3. The method of Claim 1 wherein the amount of free biological target is determined by a method comprising the steps of:
  - 25 (i) contacting the biological sample or a fraction thereof with a saturating amount of a quantifiable irreversible inhibitor of the biological target, so that substantially all of the free biological target reacts with the quantifiable irreversible biological target inhibitor to form a target/inhibitor complex; and
  - (ii) determining the amount of target/inhibitor complex formed in step (i).
4. The method of Claim 1 wherein the biological target is an enzyme, a g-  
30 protein coupled receptor, a cytokine, or a receptor kinase.
5. The method of Claim 4 wherein the biological target is MetAP-2.
6. A method for determining the extent of inactivation of MetAP-2 in a  
35 biological sample or fraction thereof derived from a subject, comprising the steps of:

(a) administering a test compound to the subject, wherein any MetAP-2 in the body of the subject that reacts with the test compound is inactivated MetAP-2 and any MetAP-2 that does not react with the test compound is free MetAP-2;

(b) removing a biological sample from the subject, wherein said biological sample

5 comprises one or more types of cells; and

(c) determining the amount of free MetAP-2 in the biological sample or a fraction thereof; and

(d) comparing the amount determined in step (c) with the amount determined in a control sample;

10 wherein a decrease in the amount determined in step (c) compared to the amount determined in step (d) is a measure of the extent of inactivation of MetAP-2 in the biological sample or fraction thereof.

7. The method of Claim 6 wherein the amount of free MetAP-2 is

15 determined using a method comprising the steps of:

(i) contacting at least a portion of the biological sample with a saturating amount of a quantifiable irreversible MetAP-2 inhibitor, whereby substantially all of the free MetAP-2 in the biological sample reacts with the quantifiable irreversible Metap-2 inhibitor to form a MetAP-2/inhibitor complex; and

20 (ii) determining the amount of MetAP-2/inhibitor complex produced in step (i).

8. The method of claim 1 wherein the biological sample is selected from the group consisting of whole blood, a blood fraction, erythrocytes, white blood cells, T-cells, B-cells, macrophages; tumor tissue; cancer cells; bone marrow; synovium,

25 synovial fluid, cerebrospinal fluid; liver tissue; brain tissue; prostate tissue, breast tissue, lymph node tissue and spleen.

9. The method of claim 1 further including the step of lysing the cells following step (b).

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10. The method of claim 1 further comprising the step of homogenizing the biological sample or a portion of the biological sample following step (b).

11. The method of Claim 6 wherein the test compound inhibits MetAP-2

35 activity *in vitro*.

12. The method of Claim 11 wherein the test compound is an irreversible inhibitor of MetAP-2.

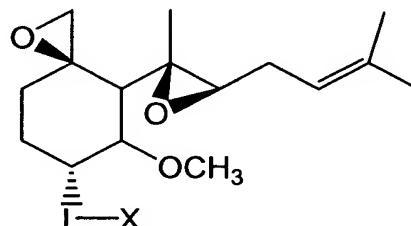
13. The method of Claim 12 wherein the test compound is a covalent 5 inhibitor of MetAP-2.

14. The method of Claim 13 wherein the test compound is a fumagillin analogue.

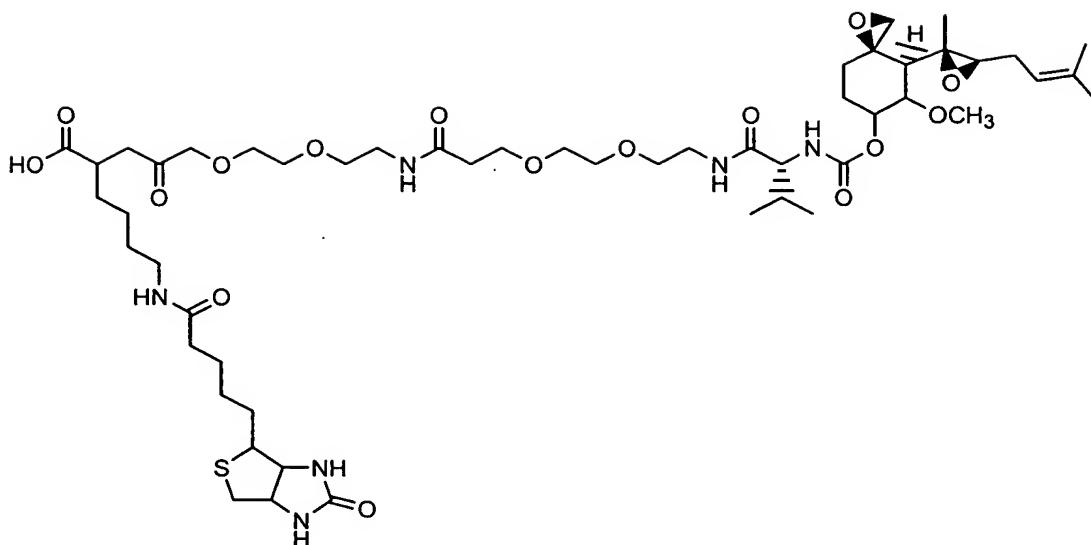
10 15. The method of Claim 1 wherein the quantifiable irreversible MetAP-2 inhibitor is a fumagillin analogue.

16. The method of Claim 15 wherein the fumagillin analogue comprises a 15 biotin moiety.

17. The method of Claim 16 wherein the fumagillin analogue is of the structure:



20 18. The method of Claim 17, wherein the fumagillin analogue is of the structure:



19. A method of quantifying a compound or compounds which are irreversible inhibitors of a biological target in a biological sample, said method comprising the steps of

5 (a) contacting the biological sample with a saturating amount of the biological target, whereby substantially all of the compound or compounds which are irreversible inhibitors of the biological target react with the biological target, thereby forming inactivated biological target and free biological target; and

10 (2) determining the amount of free biological target in the biological sample.

20. The method of Claim 19 wherein the amount of free biological target is determined by measuring the activity of the biological target.

15 21. The method of Claim 20 wherein the activity is enzymatic activity or binding activity.

22. The method of Claim 19 wherein the amount of free biological target is determined by a method comprising the steps of:

20 (i) contacting the biological sample with a saturating amount of a quantifiable inhibitor of the biological target, whereby substantially all of the free biological target in the biological sample reacts with the quantifiable irreversible inhibitor to form a target/inhibitor complex;

(ii) determining the amount of target/inhibitor complex produced in step (i); and

(iii) comparing the amount of target/inhibitor complex determined in step (i) with the total amount of biological target added in step (1),  
wherein a decrease in the amount of target/inhibitor complex determined in step (ii) compared to amount of biological target added in step (1) indicates the amount of a  
5 compound or compounds in the biological sample which are irreversible inhibitors of the biological target.

23. The method of Claim 19 wherein the biological target is MetAP-2.

10 24. The method of Claim 23 wherein the compound or compounds which are irreversible inhibitors of MetAP-2 are fumagillin analogues.

25. The method of Claim 22 wherein the biological target is MetAP-2 and the quantifiable inhibitor is a fumagillin analogue comprising a quantification moiety.

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